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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group:	1651
Confirmation No.:	7977
Application No.:	10/634,292
Invention:	**Nano-Structured Polymers For Use As Implants**
Applicant:	Haberstroh et al.
Filed:	August 5, 2003
Attorney Docket:	3220-73239
Examiner:	Davis, Ruth A.

Certificate Under 37 CFR 1.8(a)

I hereby certify that this correspondence is being deposited via facsimile transmission to the United States Patent and Trademark Office (Central Fax Division), fax number 571.273.8300.

on November 27, 2007

(Signature)

John P. Breen
Printed Name

DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. THOMAS J. WEBSTER

Mail Stop RCE
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22213-1450

I, Thomas J. Webster, declare as follows:

1. I am currently an Associate Professor for the Divisions of Engineering and Orthopedic Surgery at Brown University, and the director of the Nanomedicine Laboratory which designs, synthesizes, and evaluates nanomaterials for various implant applications. I received a Bachelors Degree in Science with a concentration in chemical engineering from the University of Pittsburgh (1995) and Masters (MS) and Doctorate of Philosophy degrees (Ph.D.) in biomedical engineering from Rensselaer Polytechnic Institute (M.S., 1997; Ph.D., 2000). My research has centered on the design, synthessis,

and study of nanophase materials for various implant applications. My lab group has generated 4 books, 33 book chapters, 85 invited presentations (including tutorials), 215 literature articles and/or conference proceedings, and 245 conference presentations.

2. I have read and understand the specification of the captioned application and the pending claims in the application. The pending claims of the captioned patent application are directed to a nano-structured synthetic implant comprising a polymeric material having a nano-sized surface feature having at least one dimension in the range from about 25 nm to less than 100 nm. I have knowledge of the results described below because the assays described below were conducted in my laboratory under my direction.

3. An exemplary *in vitro* experiment conducted using the claimed composition is shown in Exhibit A attached to this declaration and demonstrates the unique unexpected properties associated with materials having a nano-sized surface feature having at least one dimension that is less than 100 nm. Chemically-treated and cast nanostructured poly(lactic-co-glycolic acid) (PLGA) and polyurethane (PU) films (having surface feature dimensions less than 100nm) were prepared and compared to chemically-treated and cast submicron poly(lactic-co-glycolic acid) (PLGA) and polyurethane (PU) films (having surface feature dimensions of 100 nm to 1 μm) to determine the relative protein bioactivity of the two surfaces.

4. The results shown in Exhibit A show greater competitive fibronectin and vitronectin adsorption as well as RGD exposure in proteins on both chemically-treated and cast nanostructured PLGA and PU compared to respective conventional (untreated) and sub-micron polymers (Figure 1 for PLGA and Figure 2 for PU). Sub-micron polymers were observed to have the same degree of protein adsorption and bioactivity as conventional polymers. Importantly, fibronectin and vitronectin are essential cell adhesive proteins and RGD is a key cell adhesive domain in proteins. In this manner, this study demonstrates that nanostructured (either chemically-treated or cast) PLGA and PU

can promote bioactive protein adsorption and conformation more than sub-micron or conventional PLGA and PU.

All statements herein made of my own knowledge are true, and all statements herein made on information and belief are believed to be true; these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Dated: November 21 , 2007 By:

Thomas J. Webster, Ph.D.

Exhibit A

Nano-Structured Polymers For Use As Implants

Comparison of protein bioactivity on nano-structured PLGA/PU relative to sub-micron structured PLGA/PU.

METHODS

Substrate fabrication

Treated nanostructured poly(lactic-co-glycolic acid) (PLGA) and polyurethane (PU) films

PLGA samples were prepared by dissolving (at 60°C) 0.5 g of PLGA (50:50 wt.%; 12-16.5 X 10^3 MW; Polysciences, Inc., Warrington, PA) in 8 mL of chloroform (Mallinckroft, Paris, KY) for 40 min. This solution was poured into glass Petri dishes, allowed to sit overnight, and then transferred to a vacuum (15 in. Hg) oven for 2 days at room temperature. Some of the resulting polymer films were left untreated (labeled conventional), while others were treated with 10 N NaOH for 1 h (labeled nanostructured or having surface feature dimensions less than 100 nm [1]) or 5 N for 30 min (labeled sub-micron or having surface feature dimensions 100 nm to 1 μm [1]). The resulting 0.5-cm thick films were then cut using a scalpel into 1 X 1 cm strips or 4.76 mm diameter disks (for use in 96-well plates) and were sterilized by soaking in ethanol for 24 h before experiments with cells.

Polyurethane (PU; Tecoflex) films were created in a similar fashion, with the exception that nanostructured PU was created by treating with 10 N HNO_3 for 30 minutes and sub-micron PU was created by treating with 0.1 N HNO_3 for 10 minutes.

Cast nanostructured poly(lactic-co-glycolic acid) (PLGA) and polyurethane (PU) films

Casts of each PLGA and PU film created as previously described were created. Briefly, the nanostructured and sub-micron topography was captured from the previously mentioned NaOH treated PLGA and HNO_3 treated PU by using a well-established silastic casting process. Silastic (SYLCARD 184 Silicone Elastomer; Dow Coming) was poured into a Petri dish containing respective polymers, prepared as previously described. The elastomer was then allowed to cure for 48 h. The resulting negative cast was removed from the PLGA/PU, inverted, placed in a larger Petri dish and more silicone elastomer was added in order to form a well. PLGA/PU dissolved as described above was poured into the silastic mold, and the solvent was evaporated in the same manner as outlined above. These resulting 0.5-cm-thick films were then cut into 1 X 1 cm squares or 4.76 mm diameter disks (for use in 96-well plates). The substrates were then sterilized by soaking in ethanol for 24 h before experiments.

Exhibit A

Protein adsorption

ELISA

PLGA and PU substrates were separately exposed to 200 µl of Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, which contains many proteins including fibronectin and vitronectin) and 1% penicillin/streptomycin (P/S; Hyclone). The substrates were then rinsed with PBS, blocked with 2% bovine serum albumin (BSA; Sigma) for 1 h, and incubated with anti-bovine vitronectin (1100; Accurate Chemical) or anti-bovine fibronectin (1:100; Chemicon, Temecula, CA) for 1 h. Immediately thereafter, the substrates were rinsed with Tris buffered saline-0.1% Triton X-100 (Sigma) and incubated with horse radish peroxidase conjugated anti rabbit secondary antibody (1:100; Bio-Rad). An ABTS (2,2'-amino-bis (3-ethylbenthialoline-6-sulforic acid)) soluble substrate kit (Vector Labs, Burlingame, CA) was used to detect secondary antibodies spectrophotometrically (Spectro MAX 190, 488 nm, Molecular Devices) per the manufacturer's instructions.

Bioactive peptide exposure

In addition, after some of the samples mentioned above were allowed to adsorb proteins contained in 10% FBS, they were incubated with antibodies to RGD (arginine, glycine, aspartic acid, a well known cell binding regions contained in proteins). Similar experiments to those described above were followed.

RESULTS

Results showed greater competitive fibronectin and vitronectin adsorption as well as RGD exposure in proteins on both chemically-treated and cast nanostructured PLGA and PU compared to respective conventional (untreated) and sub-micron polymers (Figure 1 for PLGA and Figure 2 for PU). Sub-micron polymers had the same degree of protein adsorption and bioactivity as conventional polymers. Importantly, fibronectin and vitronectin are essential cell adhesive proteins and RGD is a key cell adhesive domain in proteins [See Thapa A., Webster T.J., Haberstroh K.M., "Polymers with nano-dimensional surface features enhance bladder smooth muscle cell adhesion," Journal of Biomedical Materials Research Part A, 67:1374 (2003).]. In this manner, this study demonstrates that nanostructured (either chemically-treated or cast) PLGA and PU can promote bioactive protein adsorption and conformation more than sub-micron or conventional PLGA and PU.

Exhibit A

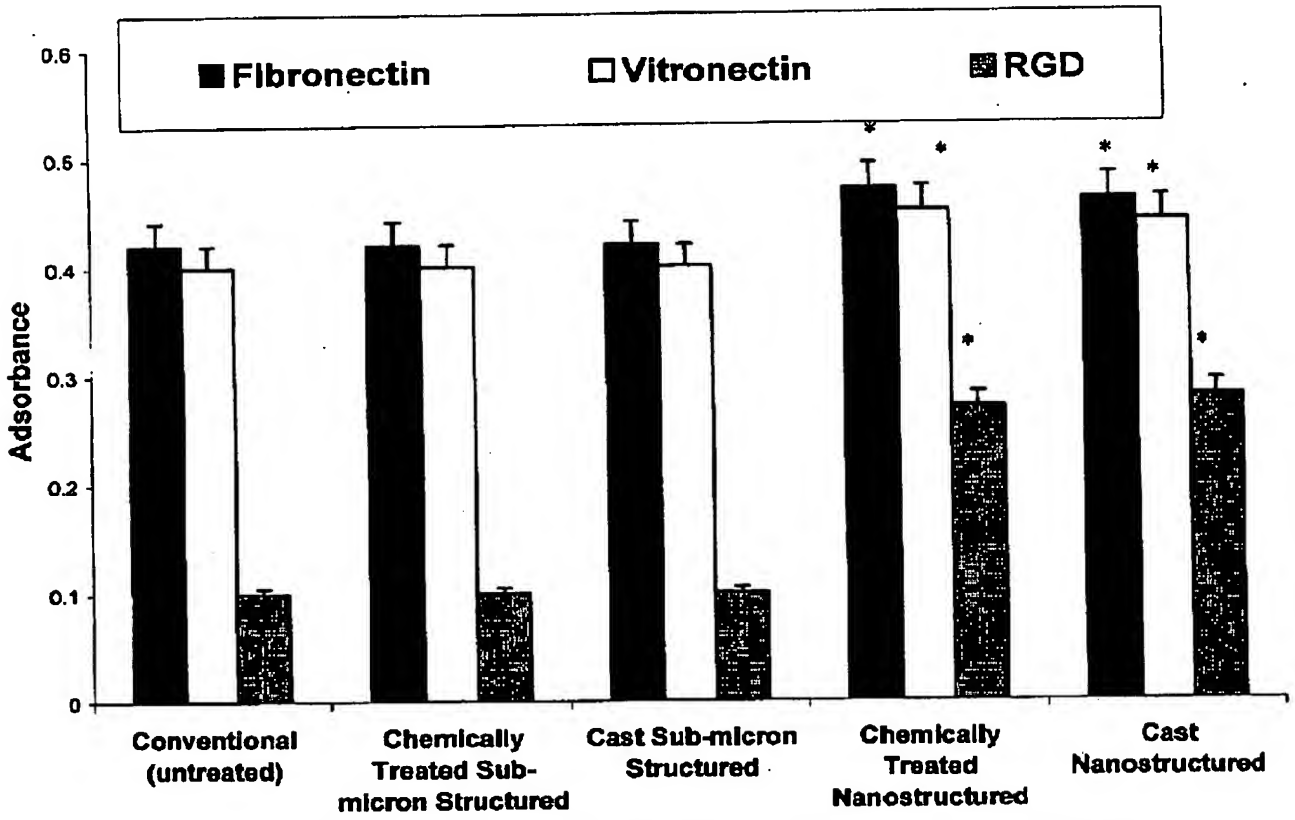

Figure 1: Increased Fibronectin and Vitronectin Adsorption as well as RGD Exposure on Nanostructured PLGA. Data = mean +/- SEM; N =3; * $p < 0.01$ compared to respective protein adsorption or RGD exposure on conventional and sub-micron PLGA.

Exhibit A

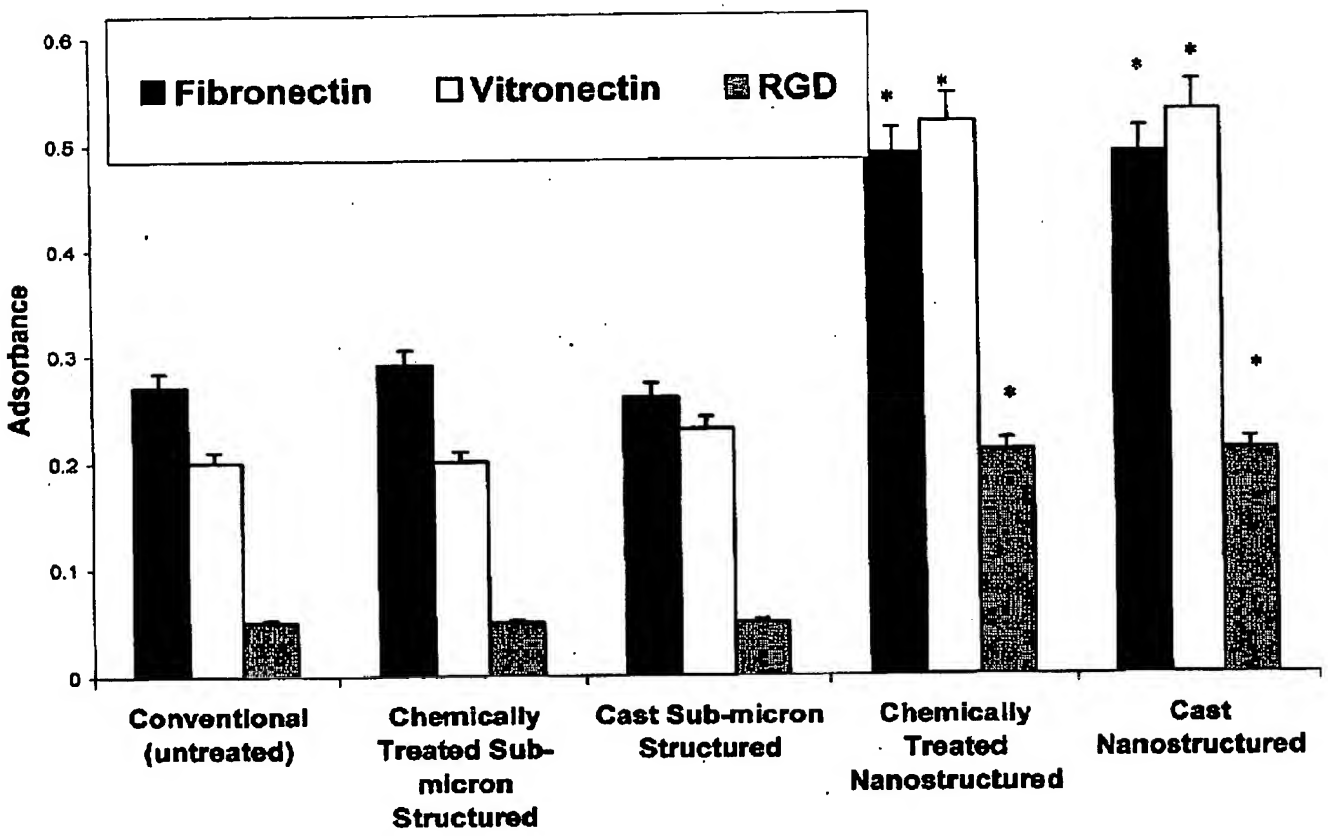

Figure 2: Increased Fibronectin and Vitronectin Adsorption as well as RGD Exposure on Nanostructured PU. Data = mean +/- SEM; N =3; * $p < 0.01$ compared to respective protein adsorption or RGD exposure on conventional and sub-micron PU.